PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 July 2000 (24.07.00)	
International application No. PCT/IL99/00649	**Applicant's or agent's file reference** 120618.4 DAB
International filing date (day/month/year) 01 December 1999 (01.12.99)	**Priority date** (day/month/year) 01 December 1998 (01.12.98)
Applicant WILLNER, Itamar et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 June 2000 (18.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
120618.4 DAB		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 99/00649	01/12/1999	01/12/1998
Applicant		
YISSUM RESEARCH DEVELOPMENT COMPANY OF ... et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
120618.4 DAB		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00649	01/12/1999	01/12/1998
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
YISSUM RESEARCH DEVELOPMENT COMPANY OF ... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/06/2000	01.03.2001
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Barz, W Telephone No. +49 89 2399 7320

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00649

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-22 as originally filed

Claims, No.:

1-45 as received on 15/02/2001 with letter of 12/02/2001

Drawings, sheets:

1/11-11/11 as originally filed

Sequence listing part of the description, pages:

1-3, filed with the letter of 17.12.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
   see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-21, 23-44
	No:	Claims 22, 45
Inventive step (IS)	Yes:	Claims 1-21, 23-44
	No:	Claims 22, 45
Industrial applicability (IA)	Yes:	Claims 1-45
	No:	Claims

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

ITEM I:

The correction of the sequence listing and the amendments to the claims fulfill the requirements of Article 34(2)(b) PCT.

ITEM V:

Reference is made to the following document:

D2: WO 92 08808 A (Siska Diagnostics Inc.), 29 May 1992;

1. NOVELTY

Claims 22, 34, and 45 do not meet the requirements of Article 33(2) PCT for the following reasons:

1.1 The expression "For use in [...]" must be construed as meaning merely "Suitable for use in [...]" (PCT Guidelines III-4.8). Therefore, the subject-matter of **claims 22 and 45** is not novel in the sense of Article 33(2) PCT, because verification oligonucleotides as well as amplifying agents suitable for the methods or systems of the present invention are well known in the art (see also item VIII. below).

1.2 The remaining **claims 1-21 and 23-44** are novel in the sense of Article 33(2) PCT, because none of the available prior art documents discloses the same combinations of features as these claims.

2. INVENTIVE STEP

Claims 1-21 and 23-44 also appear to involve an inventive step for the following reasons:

2.1 Compared to document D2 (abstract; page 7, lines 14-24; figure 1; claims 1-3), which is considered to represent the closest prior art, the subject-matter of **claim 1** differs in that the sensor device comprises an electrochemical probe carrying the sensing interface. According to the description (page 5, lines 18-28; page 7, lines 18-22), the technical effect of this difference is that the binding of the target oligonucleotide can be detected using electrochemical techniques. Therefore, the technical problem to be solved by claim 1 may be regarded as how to provide a sandwich hybridization assay using an alternative detection technique. The solution proposed in claim 1 appears to involve an inventive step (Article 33(3) PCT), because the available prior art does not provide any evidence for electrochemical detection in hybridization assays. Therefore, it would not be obvious to the skilled person to include electrochemical detection techniques into the known sandwich hybridization assay. Consequently, the method of **claim 1** as well as its dependent **claims 2-11** seems to involve an inventive step (Article 33(3) PCT).

2.2 Similarly, the subject-matter of **claim 12** and its dependent **claims 13-21** appears to involve an inventive step (Article 33(3) PCT), because the available prior art does not provide any evidence for the incorporation of an electrochemical electrode into sensor devices for detecting target oligonucleotides. Therefore, the skilled person would have no incentive to combine all the features of claims 12-21.

2.3 Compared to D2, the method of **claim 23** differs in that the signal-amplifying agent comprises a liposome. According to the description (page 7, lines 7-11; example 2), the technical effect of this difference is that liposome binding facilitates the detection of target oligonucleotide binding. Therefore, the technical problem to be solved by claim 23 may be regarded as how to provide a sandwich hybridization assay using an alternative detection technique.

Similar to the argumentation above (see item 2.1 above), the solution proposed in **claim 23** and its dependent **claims 24-33** appears to involve an inventive step (Article 33(3) PCT), because the available prior art does not provide any evidence for signal-amplifying agents comprising liposomes. Therefore, it would not be obvious to the skilled person to include liposomes into the signal-amplifying agents of known sandwich hybridization assays.

2.4 Similarly, the subject-matter of **claim 34** and its dependent **claims 35-44** appears to involve an inventive step (Article 33(3) PCT), because the available prior art does not provide any evidence for detection systems comprising a signal-amplifying agent comprising a liposome. Therefore, the skilled person would have no incentive to combine all the features of claims 34-44.

3. INDUSTRIAL APPLICABILITY

The subject-matter of **claims 1-45** is industrially applicable in the sense of Article 33(4) PCT.

4. P-DOCUMENTS

The claim to priority of the present application is valid. Therefore, the prior art document which was published after the priority date, but before the filing date of the present application (listed as "P,X" document in the International Search Report) is not relevant for the present application

ITEM VIII:

1. **Claim 22 and 45** are neither clear nor supported by the description (Article 6 PCT), because the reagents are not defined by technical features of the "verification oligonucleotide" and the "amplifying agent". Consequently, their scope is broader than justified by the description and drawings.

2. Furthermore, **claims 22 and 45** do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved ("agent for amplifying the signal [...]") which merely amounts to a statement of the underlying problem.

PATENT COOPERATION TREATY

PCT

Corrected version

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
120618.4 DAB		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 99/00649	01/12/1999	01/12/1998
Applicant		
YISSUM RESEARCH DEVELOPMENT COMPANY OF ... et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

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[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted]L 99/00649

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 695 926 A (CROS PHILIPPE ET AL) 9 December 1997 (1997-12-09) see whole doc esp. claims	1
X	WO 92 08808 A (SISKA DIAGNOSTICS INC) 29 May 1992 (1992-05-29)	1
Y	see claims and figure	2-7,16
Y	OKAHATA Y. ET AL.,: "Kinetic measurement of DNA hybridization on an oligonucleotide-immobilized 27-MHz quartz crystal microbalance" ANAL. CHEM., vol. 70, - 1 April 1998 (1998-04-01) pages 1288-1296, XP000891733 the whole document	2-7,16
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 April 2000	13/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JENSEN K K ET AL: "KINETICS FOR HYBRIDIZATION OF PEPTIDE NUCLEIC ACIDS (PNA) WITH DNA AND RNA STUDIED WITH THE BIACORE TECHNIQUE" BIOCHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, PA, vol. 36, 1 January 1997 (1997-01-01), pages 5072-5077, XP002062488 ISSN: 0006-2960 ---	
P,X	PATOLSKY F. ET AL.,: "Enzyme-linked amplified electrochemical sensing of oligonucleotide-DNA interactions by means of the precipitation of a insoluble product and using impedance spectroscopy" LANGMUIR, vol. 15, - 29 April 1999 (1999-04-29) pages 3703-3706, XP000901187 the whole document -----	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
P[redacted]L 99/00649

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5695926 A	09-12-1997	FR 2663040 A	13-12-1991
		AT 133995 T	15-02-1996
		AU 650885 B	07-07-1994
		AU 7995391 A	07-01-1992
		CA 2059657 A	12-12-1991
		DE 69116993 D	21-03-1996
		DE 69116993 T	14-11-1996
		DK 486661 T	17-06-1996
		EP 0486661 A	27-05-1992
		ES 2084167 T	01-05-1996
		FI 102296 B	13-11-1998
		WO 9119812 A	26-12-1991
		JP 5501957 T	15-04-1993
		PT 97939 A	31-03-1992
WO 9208808 A	29-05-1992	AU 6807496 A	16-01-1997
		AU 9115891 A	11-06-1992
		CA 2095611 A	15-05-1992
		EP 0557456 A	01-09-1993
		JP 6502766 T	31-03-1994
		US 5474895 A	12-12-1995

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/IL 96/00049

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	BIOSENS. BIOELECTRON. (1996), 11(6/7), 591-598 CODEN: BBIOE4;ISSN: 0956-5663, 1996, XP000612767 RICKERT, JAN ET AL: "A new affinity biosensor: self-assembled thiols as selective monolayer coatings of quartz crystal microbalances" see the whole document ---	1,2,6-8, 10,12, 13,17
P,Y	EP 0 668 502 A (YISSUM RES DEV CO) 23 August 1995 see the whole document --- -/--	3-5,11, 14-16,18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1996	0 4 -04- 1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Wells, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern. J Application No
PCT/IL 96/00049

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THIN SOLID FILMS, vol. 260, no. 2, 15 May 1995, pages 192-199, XP000511908 GEDDES N J ET AL: "SURFACE CHEMICAL ACTIVATION OF QUARTZ CRYSTAL MICROBALANCE GOLD ELECTRODES - ANALYSING BY FREQUENCY CHANGES, CONTACT ANGLE MEASUREMENTS AND GRAZING ANGLE FTIR"	1,2,6-8, 10,12, 13,17
Y	see the whole document	3-5,11, 14-16,18
X	WO 94 02852 A (DU PONT) 3 February 1994 see page 32, line 25 - line 32; examples 4-6	1,6-8,12
X	WO 89 09937 A (DU PONT) 19 October 1989 see figures 7,8; table 3	1,6-8,12
X	WO 91 05261 A (DU PONT) 18 April 1991	1,2,6-8, 10,12, 13,17
Y	see page 33, line 15 - line 23; claim 1	3-5,11, 14-16,18
Y	J. AM. CHEM. SOC. (1993), 115(11), 4937-8 CODEN: JACSAT;ISSN: 0002-7863, 1993, XP002020933 WILLNER, ITAMAR ET AL: "Photoregulated binding of spiropyran-modified concanavalin A to monosaccharide-functionalized self-assembled monolayers on gold electrodes" see the whole document	3-5,11, 14-16,18
Y	J. AM. CHEM. SOC. (1994), 116(20), 9365-6 CODEN: JACSAT;ISSN: 0002-7863, 1994, XP002020934 WILLNER, ITAMAR ET AL: "Application of Photoisomerizable Antigenic Monolayer Electrodes as Reversible Amperometric Immunosensors" see the whole document	3-5,11, 14-16,18

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 96/00049

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See annex.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 8 (in part), 10-18

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/IL 96/ 00049

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1 . Claims 1-7, 8 (in part) 10-18 : Systems, methods and probes using piezoelectric devices with treated electrodes for binding non-aqueous analytes.

2 . Claims 8 (in part), 9 : Method using a piezoelectric device for binding analyte molecules which are a priori suspended or dissolved in a gas.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. J Application No
PCT/IL 96/00049

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0668502 A	23-08-95	JP 7301615 A	14-11-95
WO 9402852 A	03-02-94	CA 2139242 A	03-02-94
		EP 0650598 A	03-05-95
		JP 7509264 T	12-10-95
WO 8909937 A	19-10-89	US 4999284 A	12-03-91
		CA 1332221 A	04-10-94
		DE 68916423 D	28-07-94
		DE 68916423 T	24-11-94
		EP 0408578 A	23-01-91
		JP 3503567 T	08-08-91
WO 9105261 A	18-04-91	CA 2066643 A	05-04-91
		DE 69010506 D	11-08-94
		DE 69010506 T	22-12-94
		EP 0494896 A	22-07-92
		JP 5500715 T	12-02-93

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/543

A3

(11) International Publication Number: WO 97/04314

(43) International Publication Date: 6 February 1997 (06.02.97)

(21) International Application Number: PCT/IL96/00049

(22) International Filing Date: 16 July 1996 (16.07.96)

(30) Priority Data:
114692 21 July 1995 (21.07.95) IL

(71) Applicant (*for all designated States except US*): YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [IL/IL]; 46 Jabotinsky Street, 92182 Jerusalem (IL).

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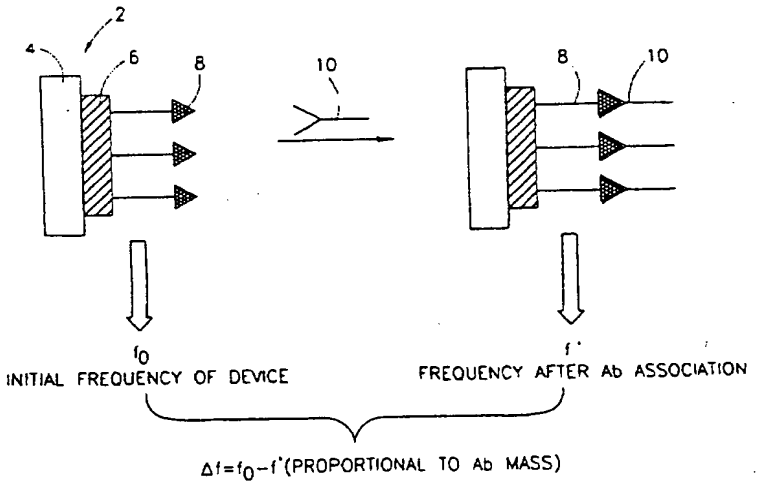

(57) Abstract

Binding between two members of a recognition pair, e.g. antigen-antibody is determined by utilizing a probe comprising a piezoelectric crystal with electrodes on two opposite faces of the crystal. The crystal carries one or more metal plates which may be the same or different than said electrodes, the metal plates having immobilized thereon a first member of a recognition pair. Binding of a second member of the recognition pair to the first member, or dissociation between the two members and release of the second member from the probe, causes a change of immobilized mass which results in a change to the probe's resonance frequency. Said immobilized members may be immobilized on the surface of said metal plates by means of a linking group, having the following general (I): Z-R^1-Q wherein Z represents a sulphur-containing moiety which is capable of chemical association with, attachment to or chemisorption onto said metal, R^1 represents a connecting group; Q is a functional group which is capable of forming a covalent bond with a moiety of said first member of the recognition pair. The immobilized member may have or be linked to an isomerizable group which changes its isomerization state as a result of exposure to energy.

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(54) Title: METHOD AND SYSTEM FOR DETECTING OLIGONUCLEOTIDES IN A SAMPLE

(57) Abstract

The present invention provides a method and a system for the detection of a target oligonucleotide in a sample. The method comprises, in general, the steps of providing a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotide; providing verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than the first portion; contacting the sample with the sensing interface under conditions so as to allow the target oligonucleotides, if present in the sample, to hybridize to the capturing oligonucleotides; prior to contacting the sample with the sensing interface or thereafter, allowing the verification oligonucleotides to hybridize to the target oligonucleotides if present in the sample; and detecting the presence of the verification oligonucleotides on the sensing interface. The detection of the verification oligonucleotides being indicative to the presence of the target nucleotide in the sample.

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METHOD AND SYSTEM FOR DETECTING OLIGONUCLEOTIDES IN A SAMPLE

FIELD OF THE INVENTION

The present invention relates to a method and system for the detection of oligonucleotides in a sample.

PRIOR ART

The following is a list of prior art references which are relevant for a better understanding of the background of the invention:

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12. PCT Application No. WO 97/04314.

Acknowledgement of these references in the description below will be made by indicating the number from the above list.

BACKGROUND OF THE INVENTION

The development of DNA-sensor devices attracts substantial recent research efforts directed to gene analysis, detection of genetic disorders, tissue matching and forensic applications. Optical detection of DNA was accomplished by the application of fluorescence labeled oligonucleotides[1,2] or by the use of surface plasmon resonance[3,4]. Electronic transduction of the formation of oligonucleotide complexes with a target DNA, and, particularly, in the quantitative assay of DNA is a major challenge of bioelectronics[5]. The organization of DNA-sensors requires the assembly of the sensing interface on a transducer, and the design of the appropriate electronic output that signals the formation of the recognition complex with the target DNA-analyte on the transducer element. Electrochemical DNA sensors based on the electrostatic attraction of electroactive transition metal complexes or organic dyes to oligonucleotide-DNA ds-complexes, e.g. $Co(bpy)_3^{3+}$, acridin or Hoechst 33258 were reported[6-10]. Microgravimetric quartz-crystal- microbalance, QCM[11] analyses were also applied to sense the formation of complementary oligonucleotide-DNA complexes.

Two major difficulties are still encountered in the development of DNA sensors and relate to the sensitivity and specificity of the resulting sensing systems.

GENERAL DESCRIPTION OF THE INVENTION

It is an object of the invention to provide a method and system for detecting target oligonucleotides in a sample.

The term *"detect"* or *"detection"* refers collectively to both a qualitative determination of the presence of the target oligonucleotide in the sample as well as at times for evaluation of the level of the target oligonucleotide in the sample.

In accordance with the first aspect of the invention there is provided a method for detecting a target oligonucleotide in a sample, comprising:

(a) providing a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotide;

(b) providing verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion;

(c) contacting the sample with the sensing interface under conditions which allow the target oligonucleotides, if present in the sample, to hybridize to the capturing oligonucleotides;

(d) prior to (c) or thereafter, allowing the verification oligonucleotides to hybridize to the target oligonucleotides if present in the sample; and

(e) detecting the presence of said verification oligonucleotides on the sensing interface.

In accordance with another aspect, the present invention provides a system for detecting a target oligonucleotide in a sample, comprising:

(i) a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotides;

(ii) verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion; and

(iii) a combination comprising one or both of apparatus and reagents for detecting a verification oligonucleotide bound to the sensing interface.

The sample may be a biological specimen or a fractionation product thereof containing the oligonucleotides; a biological specimen treated to free and solubilized oligonucleotides; a specimen treated in a manner so as to digest nucleotide sequence into smaller oligonucleotides; a sample of oligonucleotides obtained by a PCR (Polymer Chain Reaction) process or any other oligonucleotide amplification process; etc.

In accordance with one embodiment, the present invention may be applied for a variety of genetic screening assays, such as, for example, screening intended to locate mutant genes.

In accordance with another embodiment, the invention may be applied for identifying pathogens in a sample.

There is a wide variety of assaying techniques available for detecting oligonucleotides which are based on hybridizing a probe oligonucleotide to the target oligonucleotide. Also known are assay techniques wherein a probe oligonucleotide is bound to a solid support which hybridize and *"fish out"* the target oligonucleotide from a tested sample. The invention is however unique in that it makes use of a verification oligonucleotide which increases both specificity and sensitivity of the assay.

In accordance with the invention, the verification oligonucleotide serves as an indicator for the presence of the target oligonucleotide in the sample. In other words, detection of an immobilized verification oligonucleotide on the surface is an indication that the target oligonucleotide is bound to the sensing surface and hence that it existed in the sample. In accordance with the invention there are thus two discrimination means to ensure specificity and sensitivity:

1. Hybridization of the target oligonucleotide to the capturing oligonucleotide on the sensing surface. The complementary sequence of the capturing oligonucleotide will typically, but not exclusively, comprise a number of oligonucleotides completing about one helix of the nucleotide strand, i.e. about twelve nucleotides. A complementary

sequence of twelve oligonucleotides ensure on the one hand stable hybridization. On the other hand, a 12-mer oligonucleotide decreases the chance of binding to an incorrect oligonucleotide than a longer sequence. In the case the sample is a digested specimen of genomic DNA, or a fractionation product thereof comprising the oligonucleotides, there is some probability, which increases with the length of the capturing oligonucleotide, of binding to an incorrect oligonucleotide, namely an oligonucleotide other than the target oligonucleotide. This probability is lower, as aforesaid in the case of a shorter oligonucleotide. A sequence of about 12 nucleotides is preferred as it is optimal as far as ensuring binding stability, on the one hand, and reducing incorrect binding on the other hand. The invention is, however, not limited to such a capturing oligonucleotide.

2. Hybridization of the verification oligonucleotide to the target oligonucleotide.

These two independent binding events thus reduce the chance of false positive or false negative results.

The detection of the verification oligonucleotide on the sensing surface may be achieved by a number of means. In accordance with one embodiment of the invention, the sensor device comprises an electrochemical probe for electrical/ electrochemical measurements, e.g. for Faradaic impedance spectroscopy measurement or amperometric detection of the oligonucleotide. In addition, detection may also be carried out by a number of other electrochemical techniques known *per se* based on the control of interfacial electron transfer rates between the sensing interface and the surrounding medium. For this electrochemical embodiment of the invention, the sensing surface is formed on a conductive matrix on which the capturing oligonucleotides are bound. Such an electrically conducting matrix may for example be made or coated by a metal such as gold, platinum, silver or copper.

In accordance with another embodiment of the invention, the sensing device is a quartz crystal microbalance (QCM) probe in which case the presence of the verification oligonucleotide on the sensing surface is based on measurement of changes in resonance frequency of the probe. Microgravimetric QCM techniques are known *per se*, and are described, for example, in PCT Application WO 97/04314[(12)].

In accordance with one preferred embodiment of the invention, the verification oligonucleotide is conjugated to a recognition agent which specifically binds to a signal-amplifying agent. The signal-amplifying agent, according to this embodiment, comprises a recognition partner capable of specific binding to the recognition agent. The recognition agent and the recognition partner constitute together a recognition couple. In accordance with another preferred embodiment of the invention the verification oligonucleotide is bound to or complexed directly with a signal-amplifying agent.

The recognition couple may, for example, be one of the couples selected from the group of biotin-avidin or biotin-streptavidin, receptor-ligand, sugar-lectin, antibody-antigen (the term *"antibody"* should be understood as referring to a polyclonal or a polyclonal antibody, to a fraction of an antibody comprising the variable, antigen-biotin binding portion, etc.). The recognition agent may be one member of the aforementioned couples, while the recognition partner may then be the other member of the recognition couple.

In accordance with one embodiment of the invention, the verification oligonucleotide comprises a first recognition agent and the signal-amplifying agent comprises a second recognition agent, with the first and the second recognition agents being the same or different, and both being capable of specific binding to a recognition partner to form a recognition couple. The recognition partner is thus capable of specific binding to both the first and the second recognition agents and thus its introduction to a sensing surface to which the verification oligonucleotide has bound, will yield binding of the signal-amplifying agent to the sensing interface. An example of a recognition partner is avidin or streptavidin, with both the first and

second recognition agents being biotin. In accordance with an embodiment of the invention, the signal-amplifying agent comprises a plurality of said second recognition agents and thereby, by a sequence of exposures of the sensing interface to said recognition partner and said signal-amplifying agent, a complex comprising two or more signal amplifying agents bound to each verification oligonucleotide on the sensing interface, may thereby be obtained to yield an increased signal amplification.

The signal-amplifying agent, according to one embodiment of the invention is a moiety or particle which directly increases the mass immobilized on the sensing surface. The signal-amplifying agent may, for example, comprise molecules, a super molecular structure, or particle, e.g. colloid particles, macromolecules, clusters or molecules, liposomes, etc. In addition, the signal-amplifying agent may also be conjugated to or complexed with a label including, but not limited to an enzyme label. In case of an enzyme label, the enzyme is of a kind that can catalyze a reaction giving rise to an insoluble product. In accordance with this embodiment, the enzyme, after the signal-amplifying agent binds to the recognition agent, is allowed to catalyze a reaction which gives rise to the insoluble product, and the product then precipitates onto the sensing surface. This product may then be detected by a variety of electric-electronic or optical detection means. In an assay carried out in accordance with the electrochemical embodiment, such a precipitate is preferably detected by the large change in electrode impedance resulting therefrom or, alternatively, it may be detected by the mass change on a piezaelectric crystal resulting in a frequency change of the crystal.

In accordance with an embodiment of the invention, a particle, serving as a single-amplifying agent by its own right, may also carry an enzyme for further amplification of the binding-associated signal. For example, a liposome used as a signal-amplification agent may be bound to or complexed with said enzyme to allow further increase in mass as a result of precipitation of the enzyme-catalyzed insoluble product on the sensing surface, and thus a further amplified binding- related signal.

The invention also provides, for use in the above method and system, one or more reagents, selected from the group consisting of:

(i) said verification oligonucleotide;

(ii) an amplifying agent for amplifying the signal resulting from binding of said verification oligonucleotide to said sensing interface.

The invention will now be described with reference to a non-limiting specific embodiment. As will no doubt be appreciated, this description is a mere illustrative example of the wider scope of the invention as defined in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a bioelectronic system in accordance with an embodiment of the invention including assembly of the sensor and its use in detection of an target oligonucleotide in a sample.

Figs. 2A-2D illustrate in somewhat more details some of the components of the system of Fig. 1 as used in the exemplary experiments: Fig. 2A shows a DNA strand covalently bound to biotin; Fig. 2B illustrates the chemical reaction, catalyzed by horseradish peroxidase (HRP), in which 4-chloro- naphthol is reacted to form an insoluble product; Fig. 2C shows the structure of the thiophosphate thymine; and Fig. 2D shows the sequence of some oligonucleotides used in the exemplary experiments.

Figs. 3A and **3B** show the impedance features, presented as Nyquist plots, of a bare electrode (curve a), after functionalization of the electrode with the capturing oligonucleotide (curve b), after binding the target DNA and the biotinylated oligonucleotide hybrid (curve c), after interaction with the avidin-HRP conjugate (curve d) and after some period of catalysis of the enzyme resulting in deposit of insoluble product on the sensing surface (curve e). It should be noted that Figs. 3A and 3B are of the same experiment but drawn to different scales.

Fig. 4A illustrates a bioelectronic system in accordance with a further embodiment of the invention including assembly of the sensor and its use in detection of a target oligonucleotide in a sample.

Fig. 4B shows the sequence of the different oligonucleotides used in a system of the kind illustrated in Fig. 4B, in the accompanying experiments.

Figs. 5A shows the impedance spectra presented as Nyquist plots of the feature illustrated in Fig. 4, the spectra including: a functionalized electrode carrying the capturing oligonucleotides (curve a); after interacting the functionalized electrode with a sample carrying the target oligonucleotide (curve b); after interaction with the oligonucleotide-functionalized liposome (curve c); after interacting an electrode functionalized with a mutated capturing oligonucleotide (curve d); and after treatment of the mutated capturing oligonucleotide bearing electrode with funcionalized liposome (curve e).

Fig. 5B shows the changes in electron transfer resistance of a functionalized electrode upon treatment with different concentrations of the target oligonucleotide and amplification with labeled liposomes.

Fig. 6 illustrates a bioelectronic system in accordance with a yet further embodiment of the invention including assembly of the sensor and its use in detection of a target oligonucleotide in a sample.

Fig. 7A shows the impedance spectra presented as Nyquist plots of the features illustrated in Fig. 6, the spectra including: a functionalized electrode (curve a); after interaction with a sample carrying the target oligonucleotide pre-treated with a biotin-labeled verification oligonucleotide (curve b); after subsequent interaction with avidin (curve c); after subsequent interaction with biotinylated liposomes (curve d); after subsequent interaction for a second time with avidin (curve e); and after a subsequent interaction for a second time with the biotinylated liposomes (curve f).

Fig. 7B shows a calibration curve which corresponds to the changes in the electron transfer resistances of the functionalized electrode upon interaction with different concentrations of the target oligonucleotide and enhancement of the detecting processes by a double-step avidin/biotinylated liposome amplification path.

Fig. 8A shows time-dependent frequency changes of oligonucleotide-bound crystal after interaction with a sample containing the target oligonucleotide (curve a);

after interaction of the resulting electrode with an oligonucleotide-labeled liposome (curve b); after functionalizing a crystal with a mutated capturing oligonucleotide (curve c); after bringing into contact the mutated oligonucleotide-labeled crystal with oligonucleotide-labeled liposome (curve d); after treating an oligonucleotide-labeled crystal with oligonucleotide-labeled liposomes (curve e).

Fig. 8B shows time-dependent frequency changes of oligonucleotide-labeled crystal after interacting with a sample containing the target oligonucleotide (Curve e); after interacting the resulting interface with avidin (curve f); after interacting the resulting assembly with biotinylated liposome (curve h); after further interacting with avidin (curve g); after further interacting with biotinylated liposome (curve i); after treating an oligonucleotide labeled crystal with a mutated oligonucleotide pre-treated with biotinylated liposomes (curve j); after treating the resulting interface (of curve j) with avidin (curve k); and after treating with biotinylated liposomes.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

The manner of assembly of the DNA sensor in accordance with an embodiment of the invention and its use, are illustrated in Fig. 1. Oligonucleotide **100**, serving as the capturing oligonucleotide, includes a first portion **102**, typically a 12-base sequence, that is complementary to a first portion of the target oligonucleotide, and a second portion **106** for binding to the electrode, e.g. a gold (Au) electrode **108**. The binding portion **106** may, for example, be a several base (e.g 5) thiosphosphate thymidine (TS) sequence, illustrated in Fig. 2C. Occasionally the two portions **102, 106** may be separated by one or more separator base-nucleotides. The electrode **108** and oligonucleotide **100** are reacted such that portion **106** binds to the surface of the Au electrode. As a result, functionalized electrode with a sensing surface **110** is formed.

A verification oligonucleotide **112** is contacted with a sample which contains the target oligonucleotide **104** whereby a partial double-stranded structure **114** is formed. This structure is then contacted with the sensing surface (step B) yielding a

bifunctional double-stranded oligonucleotide assembly **116**. It should be noted that it is possible in accordance with another embodiment of the invention to first contact the sensing surface **110** with the sample and only then bring a reagent solution which comprises the verification oligonucleotide **112** into contact with the sensing surface. This will first yield binding of the target oligonucleotide **104** (if present in the sample) to the sensing surface and then binding of verification oligonucleotide **112** to yield assembly **116**. In both cases, the presence of the verification oligonucleotide **116** on the sensing surface serves as an indication of the presence of the target oligonucleotide **104** in the sample.

The detection of the verification oligonucleotide on the sensing surface may be achieved by a number of means some of which were explained above. For example, the verification oligonucleotide may carry a label which may be detected electrically, e.g. by determining change in impedance, or electron transport between the electrode **108** and the surrounding medium. The label, by one embodiment, is an enzyme which can catalyze a reaction yielding an insoluble reaction product which precipitates on the surface's electrode thus increasing impedance. This is illustrated in step C of Fig. 1.

In accordance with one embodiment, verification oligonucleotide **112** is bound to a biotin moiety **117**. A label complex **118** which comprises an avidin **119** bound to an enzyme **120** is contacted with the sensing surface (step C) resulting in binding of complex **118** to the sensing surface. Enzyme **120** can catalyze a reaction converting a substrate (**S**) into an insoluble product (**P**) which is thus deposited on the sensing surface. Both the binding of the labeling complex **118** to the sensing surface as well as the precipitation of product (**P**) onto the sensing surface can be monitored similarly as above (i.e. change of impedance or a change of mass in the case of QCM-type measurement).

In Figs. 4A, 4B and 6, the same reference numerals as used in Fig. 1 are used for like components.

According to another embodiment illustrated in Fig. 4A, use is made with a verification oligonucleotide-modified liposome **134** that is bound to the double-stranded immobilized assembly **132,** to form an immobilized double-stranded oligonucleotide-liposome assembly **138**. The binding of the labeled verification liposome onto the sensing surface can be monitored as described above.

The sensing of the target oligonucleotide **104** in accordance with a further embodiment, can be further amplified by using a double-step avidin/biotin-labeled-liposome amplification pathway as shown schematically in Fig. 6. Functionalized electrode **110** is first hybridized with target DNA **104** pre-treated with biotin-labeled oligonucleotide **112** having a portion sequence complementary with oligonucleotide **100,** immobilized on said electrode **108,** to form bifunctional double-stranded biotinylated assembly **116**. The formed assembly is then reacted with biotinylated liposomes **142** to form a liposome containing assembly **144**. This assembly can further be reacted with avidin and additional biotinylated liposomes, to yield a multi-liposome assembly **146**.

The invention will now be further illustrated by the following example:

EXAMPLES

For clarity, in the description below the same reference numeral to those used above will be used. However, by doing so, it should not, in any way, limit the scope of the invention to the specific examples below.

It should be noted that the scheme shown in Fig. 1 can be employed for various different assays than that specifically exemplified herein. Furthermore, a similar scheme, *mutatis mutandis*, may also be used for assaying a target oligonucleotide in other assay techniques, e.g. microgravimetric QCM. In this latter case rather than electric/electronic measurements, the measurement is of change in resonance frequency of the piezoelectric crystal as a result of mass change.

Example 1 Enzyme-amplified detection of a target oligonucleotide in a sample

The sensor preparation sequence as used in the Example can be seen in Fig. 1, while the sequences of the oligonucleotides used can be seen in Fig. 2D. In Fig. 2D each oligonucleotide is identified by the reference numerals used in the example.

An 18-mer oligonucleotide **100** (SEQ ID NO: 1) which included a 12-base sequence **102** that is complementary to a part of the analyte, the Tay-Sachs (TS) mutant **104** (SEQ ID NO: 2) was used. In addition, oligonucleotide **100** included a 5-base thiophosphate thymine-TS tag **106** for its assembly on the gold (Au) electrode **108**, and a single T-base separating the tag from the sensing oligonucleotide sequence. A disc Au-electrode **108**, 0.05 cm^2, was interacted with oligonucleotide **100** (20 μM, 10 hours) resulting in the assembly of the sensing interface on the gold support (step A in Fig. 1). The resulting functionalized electrode **110** was interacted with a solution that included the target analyte, the TS-mutant sequence **104** (5.8×10^{-7} g/mL^{-1}, 4 hours), and a biotinylated verification oligonucleotide **112** (SEQ ID NO: 3, bound via the 5' end to biotin, Fig. 2D), 2×10^{-5} g/mL^{-1} (step B in Fig. 1).

Verification oligonucleotide **112** is complementary to one portion of an oligonucleotide **104** and consequently these two oligonucleotides hybridize to form a partial double-stranded structure **114**. Target oligonucleotide **104** has another sequence complementary to portion **102** of capturing oligonucleotide **100** and thus step B results in the formation of a bifunctional double-stranded DNA-oligonucleotide assembly **116**.

Sensing surface with bifunctional double-stranded DNA- oligonucleotide assembly **116** is then treated with an avidin labeled with horseradish peroxidase (HRP) (1×10^{-8} g/mL^{-1}, 3 hours) (step C in Fig. 1). HRP can catalyze the oxidation of 4-chloro-1-naphthol (**S**) by hydrogen peroxide (H_2O_2) giving rise to the formation of an insoluble product (**P**) which precipitates on the electrode. Other enzyme-substrate couples yielding an insoluble product which may be used include: alkaline

phosphatase and indoyl phosphate derivatives as substrates; glucose oxidase and tetrazolium salts as substrates; etc.

As the oligonucleotide and oligonucleotide-DNA layered assemblies are negatively charged, the electrostatic repulsion of a negatively-charged redox-probe, e.g. $Fe(CN)_6^{3-/4-}$, from the electrode support is anticipated to perturb the interfacial electron transfer. This is expected to introduce an electron transfer resistance that can be detected by Faradaic impedance spectroscopy or other electrochemical means such as reduction of the amperometric response of the electrode. The biocatalytic precipitation of the product (**P**) on the electrode is expected to further insulate the conductive support and to lead to a high interfacial electron transfer resistance or a reduction of the amperometric response of an electroactive species solubilized in the medium surrounding the electrode.

Fig. 3A shows the impedance features, using $Fe(CN)_6^{3-/4-}$ as redox- probe, presented as Nyquist plots (Z_{im} vs. Z_{re}), of the bare electrode **108** (curve a), of the functionalized electrode with the sensing surface **110** (curve b) and of the layered bifunctional double-stranded oligonucleotide-target DNA and biotinylated oligonucleotide assembly (curve c). The respective semicircle diameters correspond to the interfacial electron transfer resistnaces, R_{et}. It can be seen that the electron transfer resistance increases upon the build-up of the biotinylated oligonucleotide-DNA assembly. For example, for the functionalized electrode R_{et} = 1.1 kΩ whereas R_{et} is increased to about 2.2 kΩ upon the association of the complex **114**. These results are consistent with the fact that the negative charge increases upon the two-step organization of the assembly. This results in the enhanced electrostatic repulsion of the redox-probe, and introduces higher interfacial electron transfer resistance.

Fig. 3B shows the impedance spectra of the bifunctional double-stranded assembly consisting of the target DNA linked to the sensing interface and the biotinylated oligonucleotide, before (curve c) and after (curve d) interaction with the avidin-HRP conjugate. Upon the association of the avidin-HRP biocatalytic conjugate to the layer, a considerable increase in the electron transfer resistance is observed due

to the partial insulation of the electrode by the proteins. In the presence of H_2O_2 and the substrate (S), the biocatalytic precipitation of the product onto the electrode occurs. This insulates the conductive support, resulting in a very high increase in the electron transfer resistance, curve (e), R_{et} = 17 kΩ. It should be noted that the two parameters controlling the sensitivity of the DNA-sensing devices are the time of incubation of the functionalized-monolayer-electrode **110** with the complex **114** and more important, the time-interval used to precipitate the product by the avidin-HRP biocatalyic conjugate. Using this configuration, and upon precipitation of **P** for 40 min. it was possible to sense the target DNA **104** at a concentration of 20 x 10^{-9} g/mL^{-1}, R_{et} = 7.9 kΩ.

Control experiments show that the oligonucleotide sensing assembly has a high specificity and selectivity. Treatment of the functinalized electrode **110** with the biotinylated oligonucleotide **112** and then with the avidin-HRP conjugate **118**, but without the interaction with the target DNA **104**, yielded only a minute change in the electron transfer resistance.

In order to test the specificity of the system, the same assay was performed with a DNA fragment **104'** (SEQ ID NO: 4) that corresponds to the normal gene sequence in which the 7-based mutation leads to the TS-genetic disorder. After contact of the sensing interface with a complex between fragment **104'** and the verification oligonucleotide **112**, the system was subjected to the biocatalytic precipitation process using the avidin-HRP conjugate, using the same protocol as illustrated in Fig. 1. However, no noticeable changes in the electron's transfer resistance at the electrodes were observed, implying that the lack of formation of a complex between the capturing oligonucleotide on the sensing surface and the complex formed between the target oligonucleotide **104** and the verification oligonucleotide **112** which prevented the subsequent formation of the precipitant layer on the electrode.

Cyclic voltammetry experiments (see insert Fig. 3B) further confirm the stepwise organization of the bifunctional double-stranded complex **116**, and that the precipitation of the insulating layer formed by product **P** on the electrode, gradually

perturb the electron-transfer kinetics of $Fe(CN)_6^{3-}$. Fig. 3B inset, shows the cyclic voltammograms of $Fe(CN)_6^{3-}$ at a bare Au- electrode (curve a), upon formation of the sensing assembly **110** (curve b), and upon the formation of the double-stranded assembly **110** (curve c). The stepwise assembly of the layers is accompanied by a decrease in the amperometric response of the electrode and an increase in the peak-to-peak separation between the cathodic and anodic waves of the redox-probe. This is consistent with the enhanced electron transfer barriers introduced upon the assembly of the negatively-charged oligonucleotide assembly. Association of the avidin-HRP conjugate onto the layer (curve d), further separates the redox waves of $Fe(CN)_6^{3-}$ implying that binding of the protein insulates the electrode and perturbs the interfacial electron transfer. Biocatalytic precipitation of **P** onto the electrode insulates the conductive support, and the electrical response of the redox-probe is almost entirely blocked, (curve e). The result shown in the inset of Fig. 3B demonstrates that amperometric transduction of the formation of the complex **116**, binding of avidin-linked HRP **118**, and further precipitation of the product **P** is possible.

By some modification of the assayed scheme described above, rather than determining the formation of the insoluble product precipitates on the electrode by means of a Faradaic impedance spectroscopy, it may also be determined by means of amperometric detection, by optical means and others or by microgravimetric QCM detection, an example for the latter being provided hereinbelow.

Example 2 Liposome-amplified detection of an oligonucleotide

The sensor preparation sequence as used in the example can be seen in Fig. 4A, while the sequence of the oligonucleotides used can be seen in Fig. 4B. In Fig. 4B, each oligonucleotide is identified by the reference numeral as used in the examples.

A mercaptohexyl oligonucleotide **100** (SEQ ID NO: 5 bound to the mercaptohexyl via the 3' end) including a portion **102** that is complementary to a part of the analyte **104** (SEQ ID NO: 6) and the mercapto-derived portion **106** for its assembly as a monolayer on an Au-electrode **108** was used as a capturing agent. The

mercaptohexyl oligonucleotide **100** was assembled on the Au-electrode **108** as a monolayer, to obtain the sensing interface **110** (step A in Fig. 4A). A surface coverage of the electrode of $1.1x10^{-11}$ mole/cm^2 was determined by Tarlov's electrochemical method [Tarlov M.J. *et al.* Anal. Chem. **70**:4670 (1998)], and comparable results were obtained by QCM analyses. The resulting monolayer-functionalized electrode **110** was then brought into contact with a sample containing the target analyte, oligonucleotide **104** ($5x10^{-6}$ M, 15 hours incubation, 25°C), to yield a double-stranded assembly **132** (step B in Fig. 4A) wherein at least part (**130**) of the assembled analyte is left free for further hybridization. The resulting electrode interface was then treated with oligonucleotide-labeled liposome **134** (lipid concentration 0.2mM, 15 min. 25°C). The oligonucleotide moiety **136** (SEQ ID NO: 7, bound to a mercaptohexyl group via the 3' end, Fig. 4B) within the labeled liposome **134** is complementary to the residual base-sequence **130** of the analyte. Thus, a liposome-linked three-component double-stranded assembly **138**, consisting of the capturing agent **100**, the analyte **104**, and the liposome tagged with oligonucleotide **136**, is generated on the electrode support.

The oligonucleotide-labeled-liposome was prepared by the assembly of liposomes that are composed of phosphatidic acid, phosphatidyl choline, maleimide-phosphatidylethanolamine, cholesterol (marked with 3H-cholesterol, 45 Ci/mole) at a ratio of 79:20:1:0.1, that were modified with oligonucleotide **136** by incubation therewith for 20 hours at 4°C and purified by chromatography (Sephadex G-75). The surface coverage of the liposome with oligonucleotide **136** (50-60 oligonucleotide units per liposome) was determined by reacting the resulting liposomes with Oligreen (Molecular probe) and following fluorescence intensity of the resulting liposome suspension at λ=480nm. The size of the liposomes was determined by dynamics light-scattering and corresponded to 220±20 nm.

The oligonucleotide-labeled liposomes **134** are negatively charged in order to eliminate non-specific adsorption of the liposomes onto the sensing interface. The liposomes associated with the electrode support represent "giant" negatively charged

amplifying agents that electrostatically repel a negatively charged redox-probe stabilized in the electrolyte solution. That is, the biorecognition event between the capturing oligonucleotide **100** and the target oligonucleotide **104** is amplified by the generation of a highly-charged microenvironment that repels the electroactive probe, $Fe(CN)_6^{3-/4-}$, in solution. The electron transfer resistance produced by the assembly **138** was then assayed by Faradaic impedance spectroscopy.

Fig. 5A shows the impedance spectra (in the form of Nyquist plots, Z_{im} vs. Z_{re}) of oligonucleotide-functionalized electrode **110** (curve a) after hybridization with the target oligonucleotide **100** to form the layered double-stranded oligonucleotide assembly **132** (curve b), and after interaction with the probing oligonucleotide-labled liposome **136** to form the amplified assembly **138** (curve c). While a bare Au-electrode exhibits an electron transfer resistance of 0.5 kΩ, the associated of the capturing oligonucleotide **100** onto the conducting support increased the electron transfer resistance to 3 kΩ. This is attributed to the electrostatic repulsion of the redox label, $Fe(CN)_6^{3-/4-}$, that results in a barrier for the interfacial electron transfer. The formation of the double-stranded assembly with the target oligonucleotide increased the electron-transfer resistance to R_{et}=4.5 k Ω. This is consistent with the results presented by Example 1, hereinabove and with the fact that the higher negative charge formed on the surface as a result of hybridization, enhances the electrostatic repulsion of the electroactive species on the solution. Binding of the oligonucleotide-modified liposome **134** introduced a very high electron transfer resistance corresponding to 15 kΩ. This result is attributed to the formation of a negatively charged micro-interface upon the association of the liposome to the double-stranded assembly.

A control experiment, for the evaluation of the system's specificity, was conducted, which included an attempt to detect the presence of an oligonucleotide **104'** (SEQ ID NO: 8), that included a 6-base mutation relative to the target DNA **104**. Fig. 5A further shows the impedance spectrum of the functionalized-electrode **110** after its treatment with the mutant **104`** (curve e) and the impedance spectrum of the resulting electrode after further treatment with the

oligonucleotide-labeled liposome **134** (curve e). As may be understood from the results presented in Fig. 5A, the interfacial electron transfer resistances were almost unchanged in this control experiment, implying that the sensing interface is selective for analyses of target oligonucleotide **104**. The results also indicate that no non-specific association of mutant **104`** or of the oligonucleotide-labeled liposomes **134** on the electrode took place. This is attributed to the electrostatic repulsion existing between these components and the sensing interface.

The extent of increase in the electron transfer resistance upon the binding of the analyte-oligonucleotide, and the secondary association of the modified liposome is controlled by the bulk concentration of the analyte, as shown in Fig. 5B. the lower sensitivity limit for analyzing the analyte DNA was determined to be 1.2×10^{-12} M at a signal-to-noise value of S/N = 3.

A further control experiment, where only the oligonucleotide **136** interacted with the double-stranded assembly of the capturing oligonucleotide **100** and the target oligonucleotide **104** introduced only a small increase in the electron transfer resistance, R_{et} = 4.7 kΩ., indicating that the negatively charged liposome indeed amplified the electrostatic repulsion of the redox label.

The sensing system may be further amplified as schematically illustrated in Fig. 6, wherein the presence of target oligonucleotide **104** (SEQ ID NO:1) was detected using the negatively-charged liposomes **142** carrying the biotinylated oligonucleotide **136'** (SEQ ID NO:9 bound via the 5' end to biotin). Accordingly, oligonucleotide-functionalized electrode **110** is reacted with the target oligonucleotide (5×10^{-6} M, 15 min. of hybridization, at 25°C), pre-treated with biotinylated verification oligonucleotide **112** (SEQ ID NO:5) 1×10^{-5} M, interaction time 2 hr. 25°C), being complementary to segment **102** of the target oligonucleotide (step A, in Fig. 6). This process results in a three-component double-stranded-assembly on the electrode, consisting of the capturing oligonucleotide **100**, the analyte oligonucleotide **104** and the biotin-labeled oligonucleotide **112**. Association of avidin **118** (8 min. of incubation, step B in Fig. 6) and then the biotin-tagged-liposome **142** (8 min. of

incubation, step C in Fig. 6) resulted in the formation of a negatively-charged interface **144** that amplified the primary oligonucleotide recognition event by the electrostatic repulsion of $Fe(CN)_6^{3-/4-}$ and the enhancement of the interfacial electron transfer resistance. This sensing configuration enabled the further amplification of the biorecognition event by the multiple reaction of the resulting array with avidin and then with the biotinylated liposomes to yield a dense array of the negatively-charged liposomes. The biotin-labeled liposomes were composed of phosphatidyl choline, phosphatidylethanolamine, cholesterol (marked with 3H-cholesterol, 45Ci/mole) and biotinylated phosphatidylethanolamine with a ratio corresponding to 80:20:0.1:0.1. The average coverage of the liposomes with biotin corresponded to 550, which were purified by gel chromatography (DEAE Sephadex A-25). The size of the liposomes was determined by dynamic light scattering to be 180±40 nm.

Fig. 7A shows the impedance spectra of the array in the different steps of modification. The oligonucleotide-functionalized interface **108** exhibited an electron transfer resistance corresponding to 3 kΩ (curve a), and upon the formation of the double-stranded assembly with the analyte-DNA **104** complexed with the biotinylated oligonucleotide **112** to form immobilized biotinylated analyte **116**, the electron transfer resistance increased to R_{et} = 4.8 kΩ. (curve b). Association of avidin **119** (2.5μg/ml) to the interface **116** further increased the electron transfer resistance to 7.6 kΩ, as a result of the hydrophobic, insulating features of the protein (curve c). Association of the biotin-labeled liposome to the sensing surface 30 min. lipid concentration 0.25 mM), substantially increased the electron transfer resistance, R_{et} = 14.8 kΩ. (curve d).

The sensing of the target-DNA was further amplified by the application of a second step of association of the avidin-biotinylated liposomes under the same conditions (step D in Fig. 6), that enhanced the electron transfer resistance, respectively, to 17 kΩ and 20 kΩ (curves e and f, in Fig. 7).

In a control experiment, the sensing interface was interacted with mutant, non-complementary DNA **104′** (SEQ ID NO:4, $5x10^{-6}$M), pre-treated with

biotinylated oligonucleotide **112** and subsequently treated with avidin and the biotinylated liposome, under the same conditions. A minute increase in the electron-transfer resistance corresponding to R_{et} = 3.4 kΩ. was observed, attributed to non-specific adsorption of avidin to the sensing interface.

The increase in the electron-transfer resistance at the electrode upon binding of avidin and the biotin-labeled liposome, were controlled by the bulk concentration of the target-DNA in the sample (Fig. 7B).

Using a double-step avidin/biotin-labeled-liposome amplification pathway, target DNA concentration as low as $5x10^{-14}$ (signal to noise ratio S/N=3) was detected.

In a similar manner to that described with reference to Fig. 4A, an oligonucleotide capturing agent was assembled on an Au/quarts crystal. The functionalized interface was then hybridized with a target DNA (concentration $5x10^{-6}$M) followed by interaction thereof with the oligonucleotide-labeled liposome. Fig. 8A (solid line) shows QCM-transduction of the amplified sensing of the analyte. Interaction of the functionalized crystal with the analyte (point a) resulted in a frequency decrease of Δf = 17Hz, implying a surface coverage of the analyte corresponding to $1.2x10^{-11}$ mole/cm^2. Further reaction of the double-stranded surface with the oligonucleotide-tagged liposome (point b) resulted in a substantial decrease in the crystal frequency, Δf = -120 Hz.

Fig. 8A shows also the results of a control experiment in which the sensing interface was interacted with the mutated, non-complementary oligonucleotide ($5x10^{-6}$ M, point c) followed by treatment with the tagged liposome (point d). As shown, the crystal frequency was unchanged Δf=±2 Hz upon interaction with the non-complementary DNA. Interaction with the tagged liposome slightly altered the crystal frequency, Δf=-5 Hz. This frequency change may be attributed to minute non-specific binding of the liposome to the interface. Association of the amplified oligonucleotide-tagged liposome with the interface resulted in a frequency change of Δf=-70 Hz (point e), that allows the easy amplified detection of the target DNA also

by using microgravimetric QCM assay. The lowest sensitivity limit for the detection of the target DNA by this amplification method was estimated to be 5x10-12 M (Δf = -20 Hz, after treatment with tagged liposome).

Fig. 8B shows the results of sensing a target DNA in a sample the manner described in connection with Fig. 6, however also in this case, wherein the a capturing oligonucleotide is assembled on a Au/quarts crystal. Accordingly, first an analyte-double-stranded biotinylated system was associated wit the sensing interface which resulted in a frequency decrease of 25 Hz (curve e). Binding of avidin to the biotinylated assembly yielded a frequency change of Δf ~-50 Hz (point f). Linkage of the biotin-tagged liposomes to the system amplified the primary association of the analyte and a very high frequency change Δf~-500 Hz was observed (point g). Additional treatment of the interface with avidin, Δf~-50 Hz (point h) and then with the biotin-labeled liposome (point i) resulted in a second amplification corresponding to Δf = 690 Hz.

Treatment of the sensing interface with the biotin-labeled non-complementary DNA did not yield any significant frequency change (point j) and subsequent interaction of the resulting assembly with avidin and the biotin-tagged liposome resulted in a frequency change of about –30 Hz (points k and l, respectively). As described above, this change in frequency may be attributed to non-specific association of the liposome to the interface. Using the two-step amplification pathway, the lowest sensitivity limit for sensing the target DNA was estimated to be $1x10^{-13}$M (or $1x10^{-16}$ mole/ml), which may be further enhanced by performing additional binding steps of avidin-biotinylated liposome.

CLAIMS:

1. A method for detecting a target oligonucleotide in a sample, comprising:

(a) providing a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotides;

(b) providing verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion;

(c) contacting the sample with the sensing interface under conditions such so as to allow the target oligonucleotides, if present in the sample, to hybridize to the capturing oligonucleotides;

(d) prior to (c) or thereafter, allowing the verification oligonucleotides to hybridize to the target oligonucleotides if present in the sample; and

(e) detecting the presence of said verification oligonucleotides on the sensing interface.

2. The method of Claim 1, wherein said sensor device comprises an electrochemical probe carrying the sensing interface.

3. The method of Claim 2, wherein said detection is based on Faradaic impedance spectroscopy or amperometric measurements.

4. The method of Claim 1, wherein said sensor device comprises a microbalance quartz-crystal probe carrying the sensing interface.

5. The method of Claim 4, wherein said detection is based on a microgravimetric quartz-crystal microbalance (QCM) analysis.

6. The method of any one of Claims 1-5, wherein the sequence complementary to at least a portion of the target oligonucleotide is of about 12 nucleotides.

7. The method according of any one of Claims 1-6, wherein the verification oligonucleotide is conjugated to a recognition agent which can specifically bind to a signal-amplifying agent, and step (e) of the method comprises:

(e1) contacting the sensing interface with said signal-amplifying agent;

(e2) detecting the presence of said signal-amplifying agent on the sensing interface.

8. The method of Claim 7, wherein said recognition agent is biotin and said signal amplifying agent comprises avidin.

9. The method of any one of Claims 1-6, wherein said verification oligonucleotide is bound to or complexed with a signal-amplifying agent, and step (e) comprises detecting of presence of the signal-amplifying agent on the sensing interface.

10. The method of any one of Claims 1-6, wherein the verification oligonucleotide comprises a first recognition agent which specifically binds to a recognition partner to form a recognition couple, step (e) of the method comprises the following steps:

(e1) contacting said sensing interface with said recognition partner;

(e2) contacting said sensing interface with a signal-amplifying agent comprising a second recognition agent, which may be the same or different as the first recognition agent, which can also bind to said recognition partner; and

(e3) detecting presence of said signal-amplifying agent on said sensing interface.

11. The method of Claim 10, comprising the following step between steps (e2) and (e3):

(e2.1) repeating steps (e1) and (e2) one or more times.

12. The method of any one of Claims 7-11, wherein said signal-amplifying agent comprises an enzyme which catalyzes a reaction yielding an insoluble reaction product, and step (e) comprises:

(ea) providing conditions permitting catalytic activity of said enzyme to yield formation of said insoluble reaction product; and

(eb) detecting the presence of said insoluble reaction product on said sensing interface.

13. The method of any one of Claims 7-11, wherein said signal-amplifying agent comprises a moiety or a particle which directly increases the mass immobilized on the sensing surface, the method comprises in step (e):

(ea) detecting the presence of said moiety or particle on said sensing interface.

14. The method of Claim 13, wherein said moiety or particle is a molecule, a super molecular structure or a particle.

15. A method of Claim 14, wherein said particle is a liposome.

16. A system for detecting a target oligonucleotide in a sample, comprising:

(i) a sensor device having a sensing interface carrying capturing oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a first portion of the target oligonucleotides;

(ii) verification oligonucleotides having each a nucleotide sequence complementary in at least a portion thereof to a second portion of the target oligonucleotide, other than said first portion; and

(iii) a combination comprising one or both of apparatus and reagents for detecting a verification oligonucleotide bound to the sensing interface.

17. The system of Claim 16, wherein said sensor device is an electrochemical electrode carrying said sensing surface.

18. The system of Claim 16 or 17, wherein said apparatus is adapted for the performance of an electrochemical measurement.

19. The system of Claim 16, wherein said sensor device comprises a microbalance quartz-crystal probe carrying the sensing interface.

20. The system according to Claim 20, wherein said detection is based on a micrograviметric quartz-crystal microbalance (QCM) analysis.

21. The system of Claims 16-20, wherein said capturing oligonucleotide has a nucleotide sequence complementary to said first portion which has a length of about 12 nucleotides.

22. The system of any one of Claims 16-21, wherein the verification oligonucleotide is conjugated to a recognition agent which specifically binds to a signal-amplifying agent.

23. The system of Claim 22, wherein said recognition agent is biotin and said signal-amplifying agent comprises avidin.

24. The system of any one of Claims 16-21, wherein the verification oligonucleotide is conjugated or complexed with a signal-amplifying agent.

25. A system of any one of Claims 16-21, wherein the verification oligonucleotide is conjugated to a first recognition agent, which specifically binds to a recognition partner, the recognition partner being capable of binding also to a second recognition agent, being the same or different from said first recognition agent; the system further comprises a signal amplifying agent comprising a second recognition agent.

26. A system of Claim 24 or 25, when said first and said second recognition agents are biotin and where said recognition partner is avidin or streptavidin.

27. A system of any one of Claims 16-26, wherein said signal-amplifying agent comprises an enzyme which catalyzes a reaction yielding an insoluble reaction product.

28. A system of any one of Claims 16-26, wherein said signal-amplifying agent comprises a particle or moiety which directly increases the mass immobilized on the sensing interface.

29. A system according to Claim 28, when said moiety or particle is a liposome.

30. For use in the method of any one of Claims 1-15 or the system of any one of Claims 16-29, a reagent being at least one member of the group consisting of:

(i) said verification oligonucleotide;

(ii) an amplifying agent for amplifying the signal resulting in from binding of said verification oligonucleotide to said sensing interface.

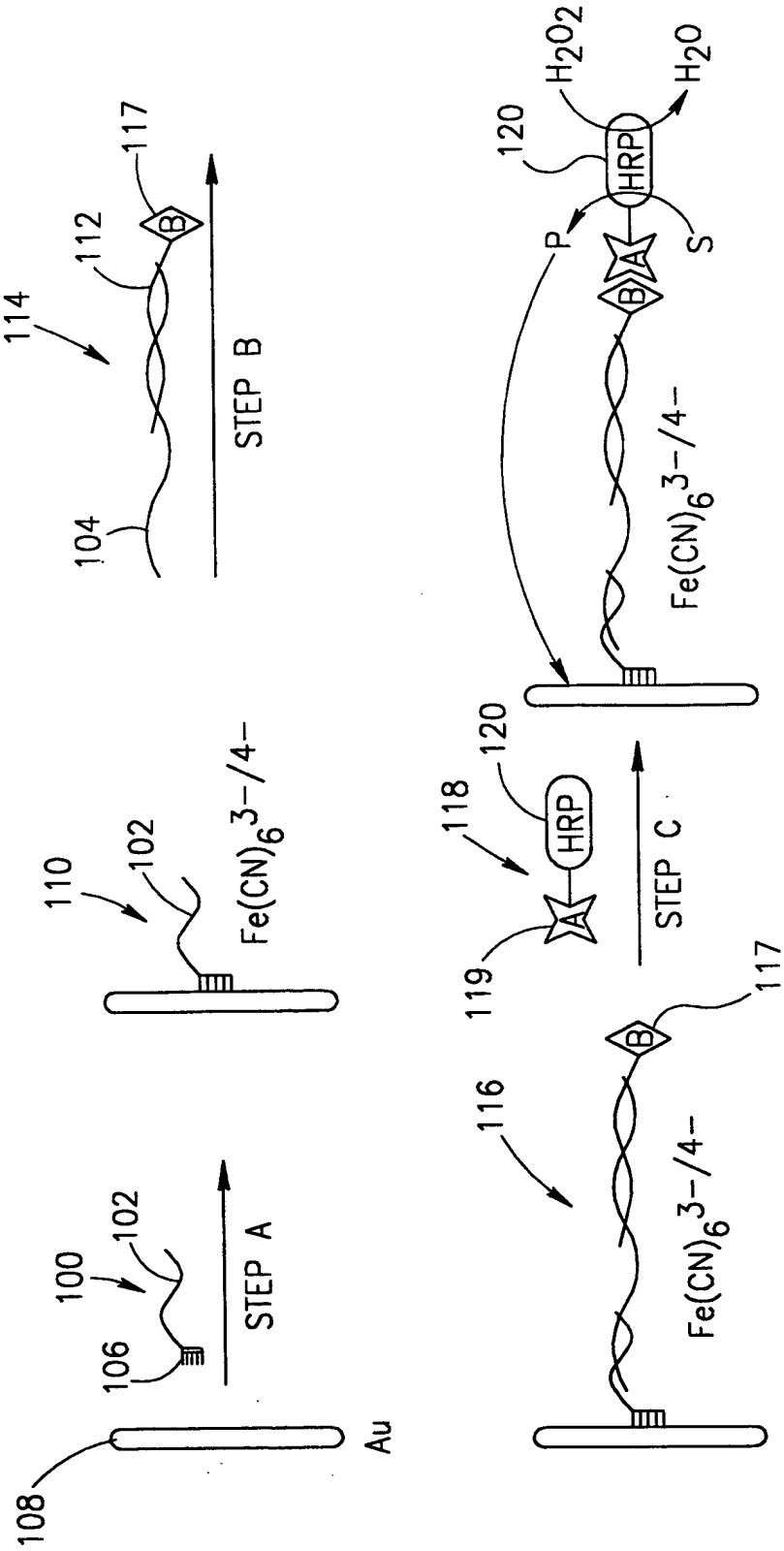

100
102
106
STEP A
108
Au
110
102
$Fe(CN)_6^{3-/4-}$
STEP B
114
112
117
104
B
116
$Fe(CN)_6^{3-/4-}$
117
B
118
120
HRP
A
119
STEP C
120
H_2O_2
H_2O
HRP
P
S
A
B
$Fe(CN)_6^{3-/4-}$

FIG.1

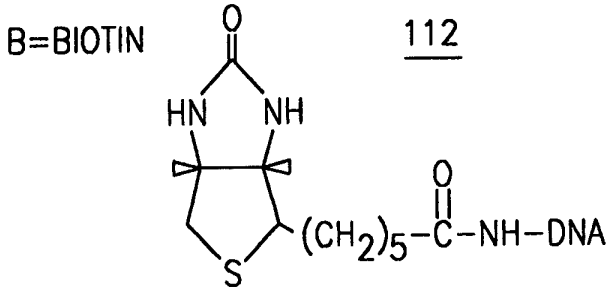


FIG.2A

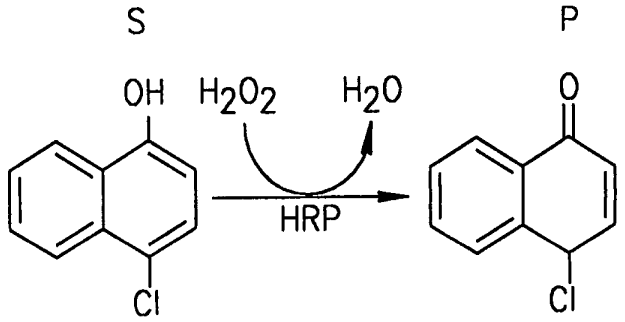


FIG.2B

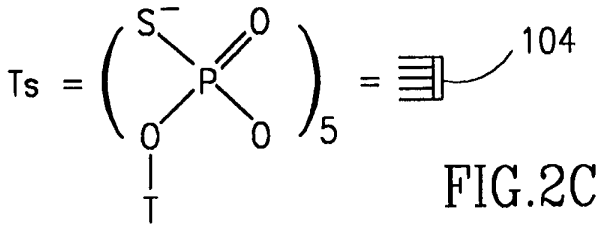


FIG.2C

100
3'TsTsTsTsTsTCGCATCCTATCT5'

104
5'GCGTAGGATAGATATACGGTTCGCGC3'

112
3'ATATGCCAAGCGCG5' –BIOTIN

104'
5'GCGGCCATAGGATATACGGTTCGCGC3'

FIG.2D

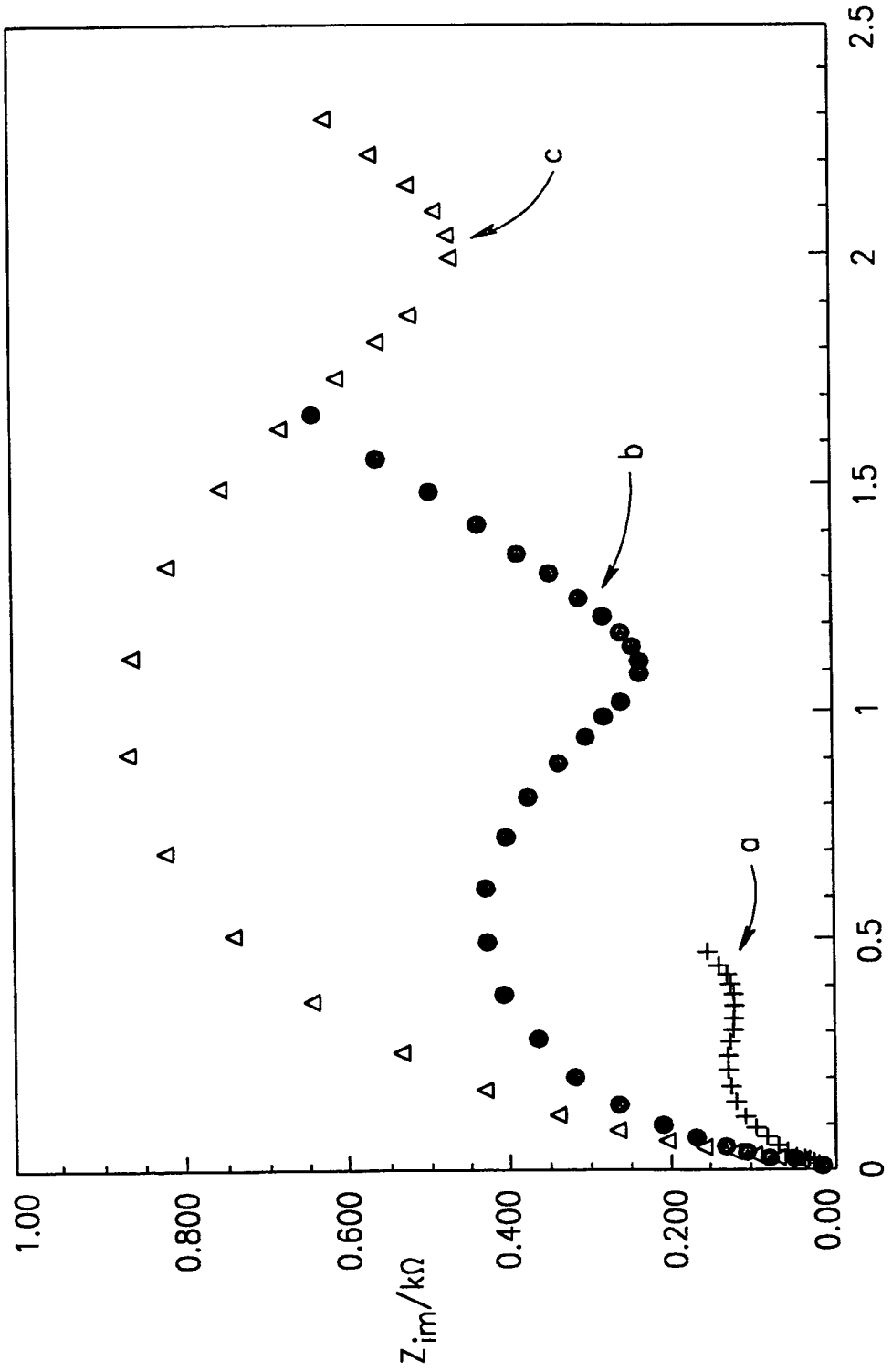

1.00
0.800
0.600
0.400
0.200
0.00
$Z_{im}/k\Omega$
0
0.5
1
1.5
2
2.5
$Z_{re}/k\Omega$
a
b
c

FIG.3A

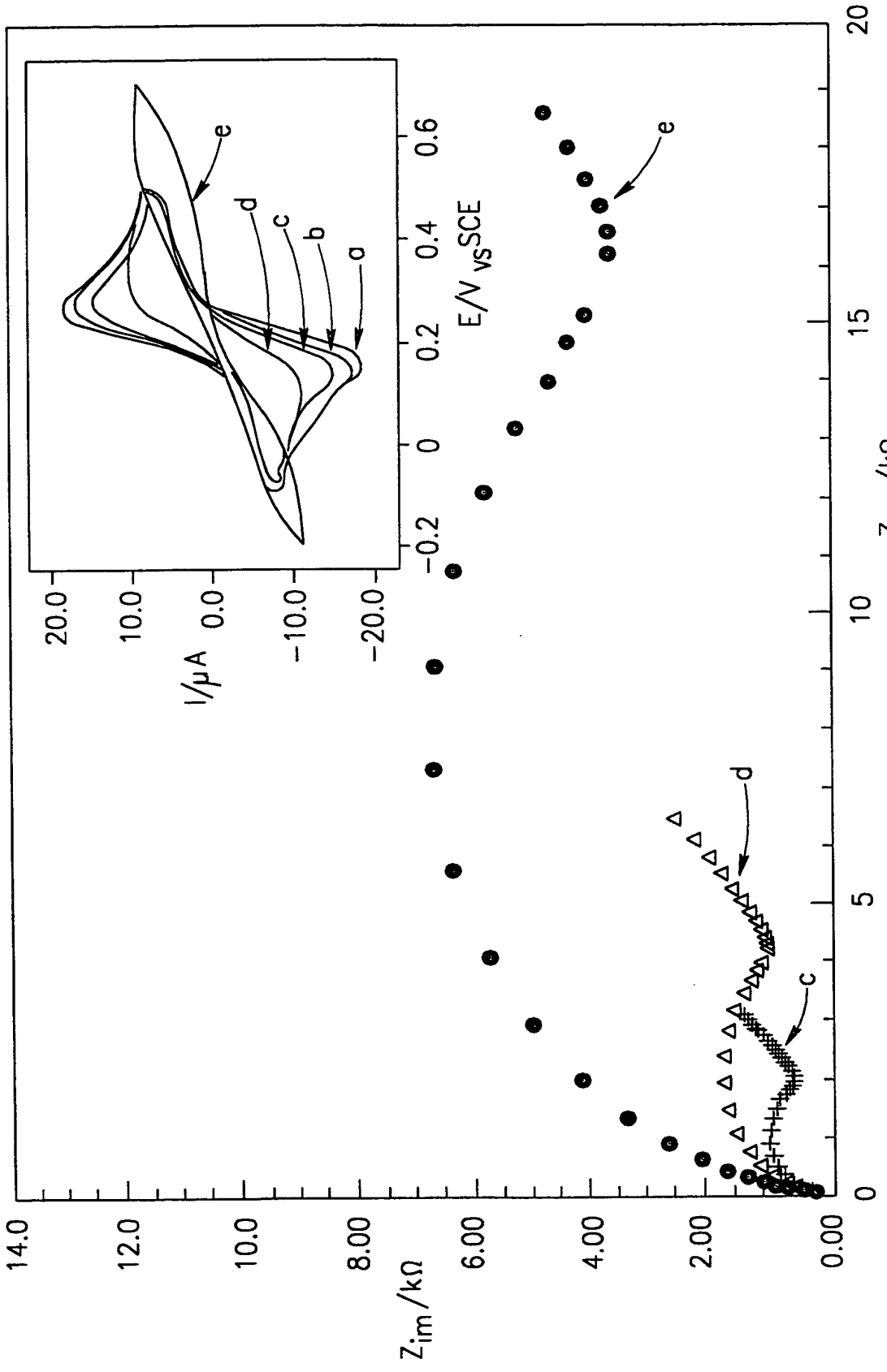


FIG.3B

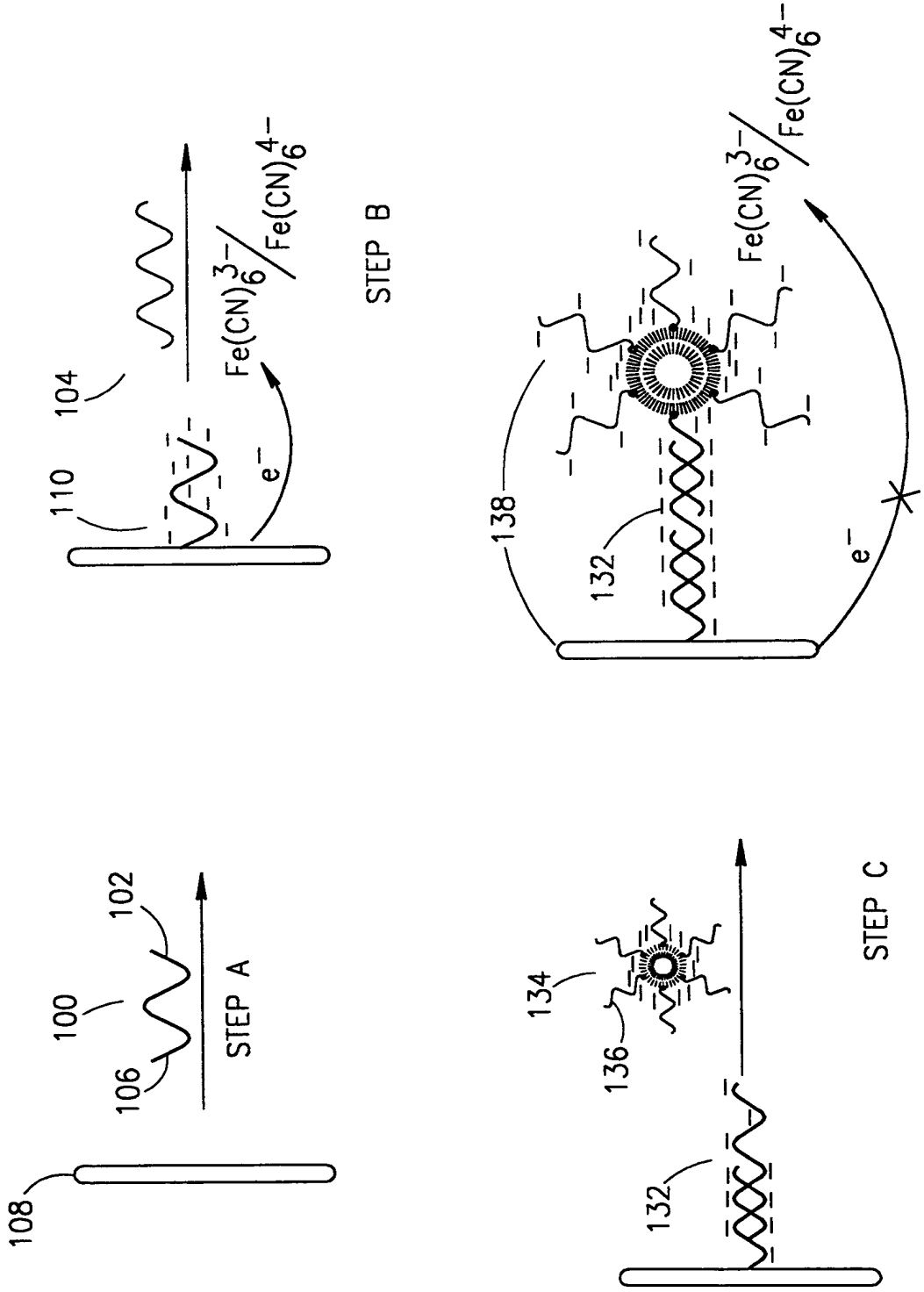

STEP A
STEP B
STEP C
100
102
104
106
108
110
132
134
136
138
e⁻
$Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$

FIG.4A

100	5'–TCTATCCTACGCT–$(CH_2)_6$–SH–3'
104	5'–AGCGTAGGATAGATATACGGTTCGCGC–3'
136	5'–HS–$(CH_2)_6$–GCGCGAACCGTATA–3'
104'	5'–AGCGCTCCAGTGATATACGGTTCGCGC–3'
136'	5'–biotin–GCGCGAACCGTATA–3'

FIG.4B

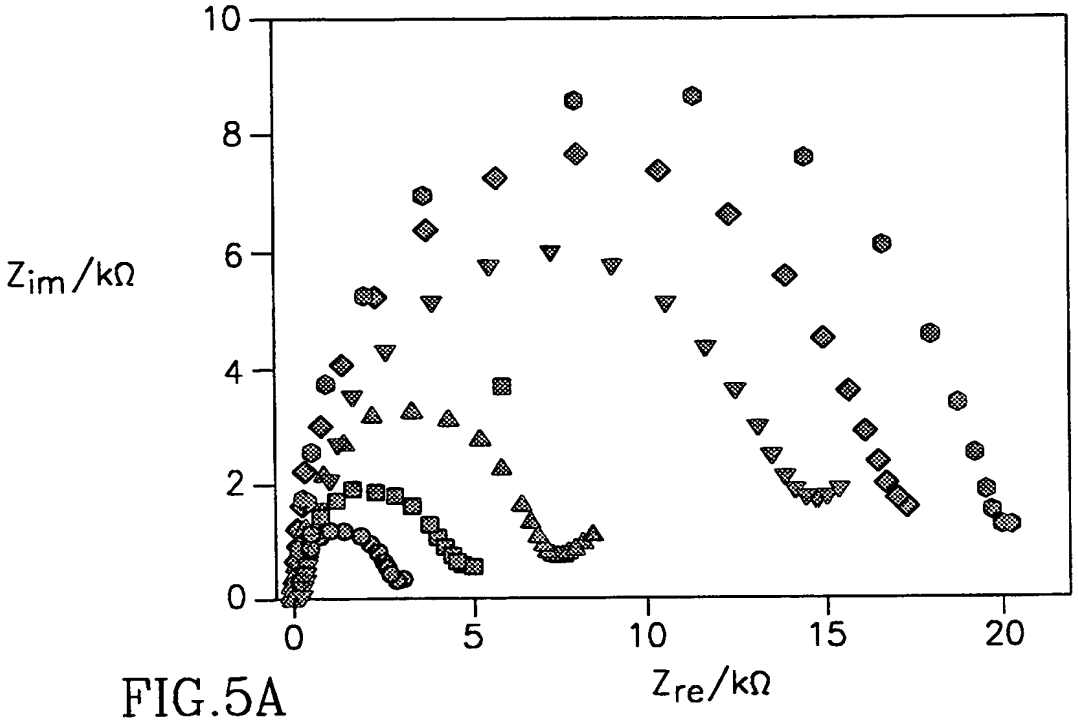

10
8
6
4
2
0
Zim /kΩ
0
5
10
15
20
Zre/kΩ

FIG.5A

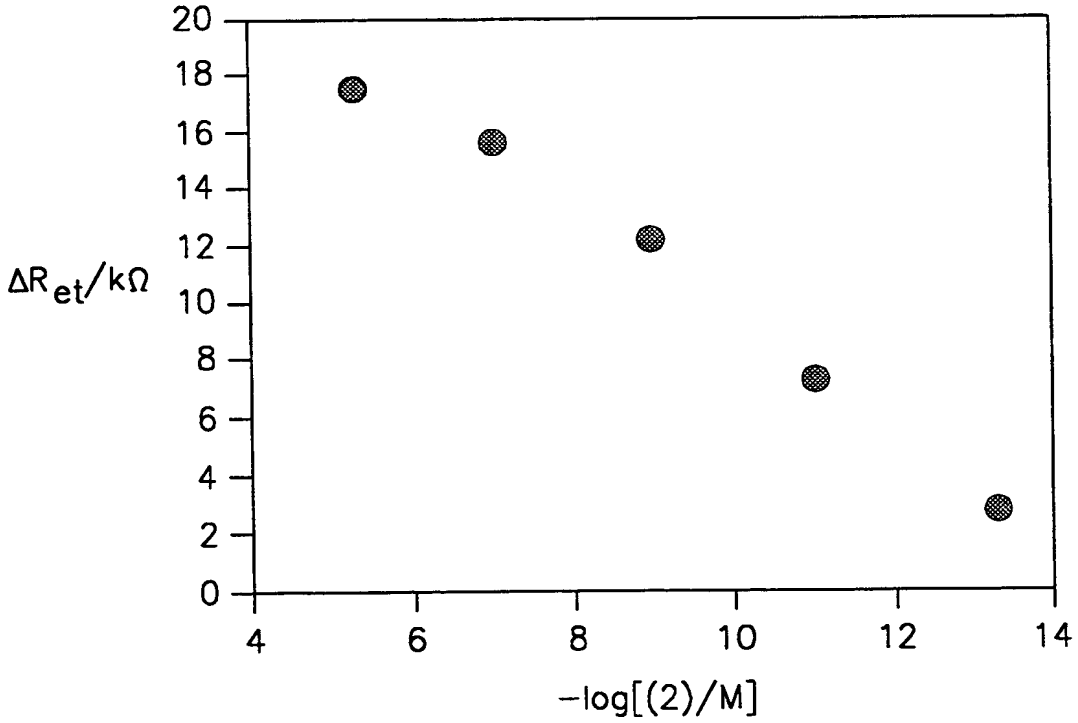

20
18
16
14
12
10
8
6
4
2
0
ΔRet/kΩ
4
6
8
10
12
14
−log[(2)/M]

FIG.5B

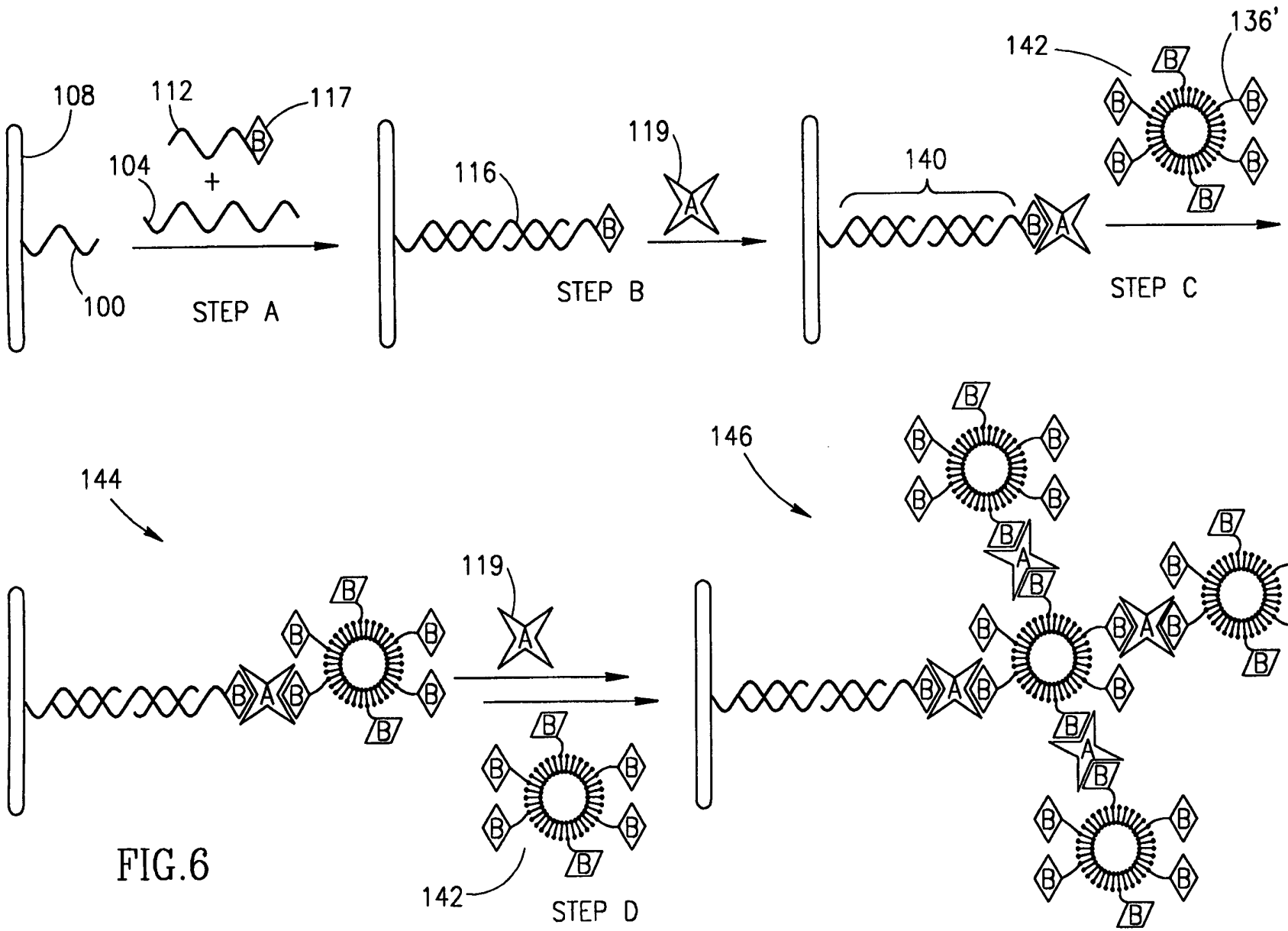

108
112
117
104
+
100
STEP A
116
119
STEP B
140
142
136'
STEP C
144
146
119
142
STEP D

FIG.6

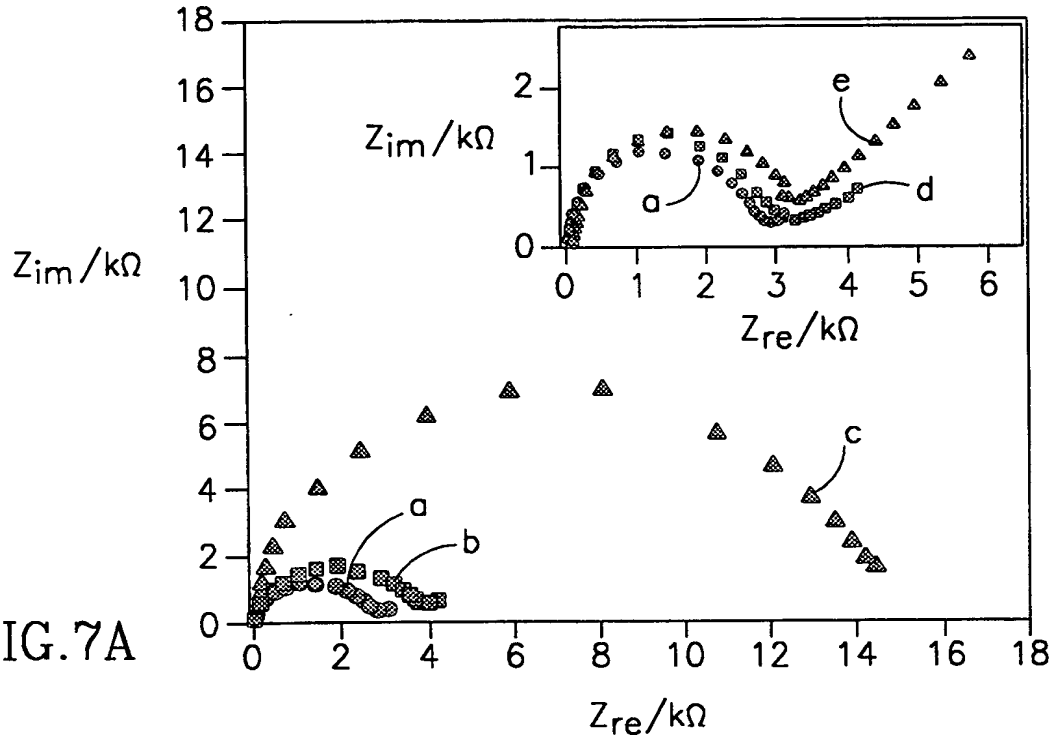


FIG.7A

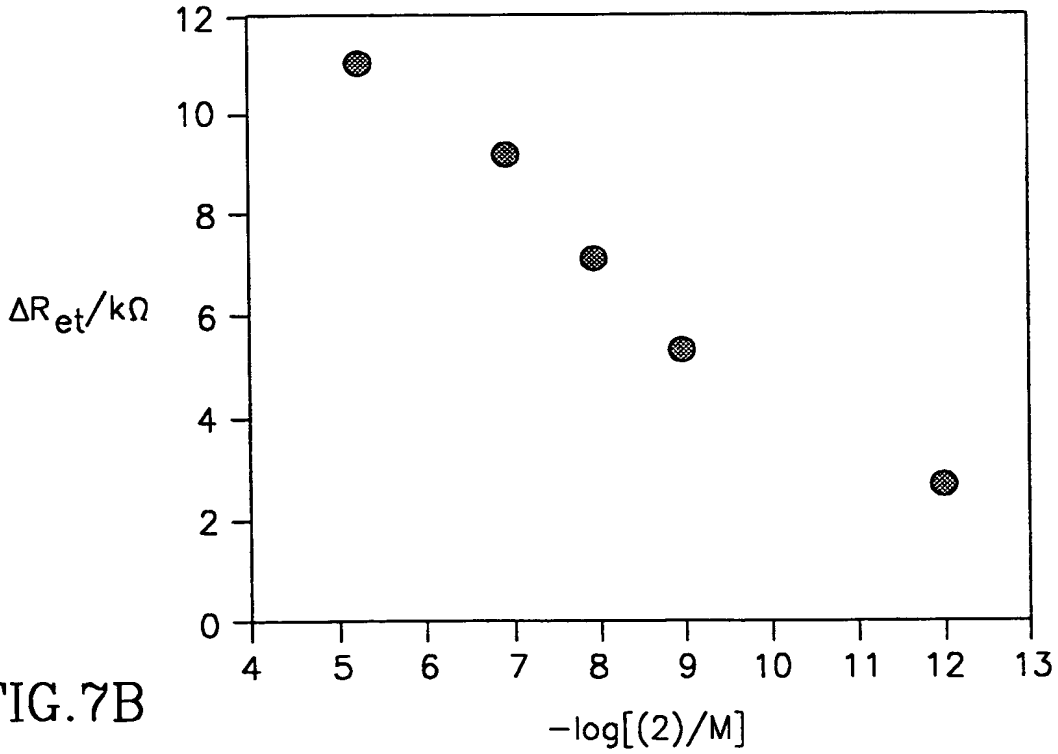
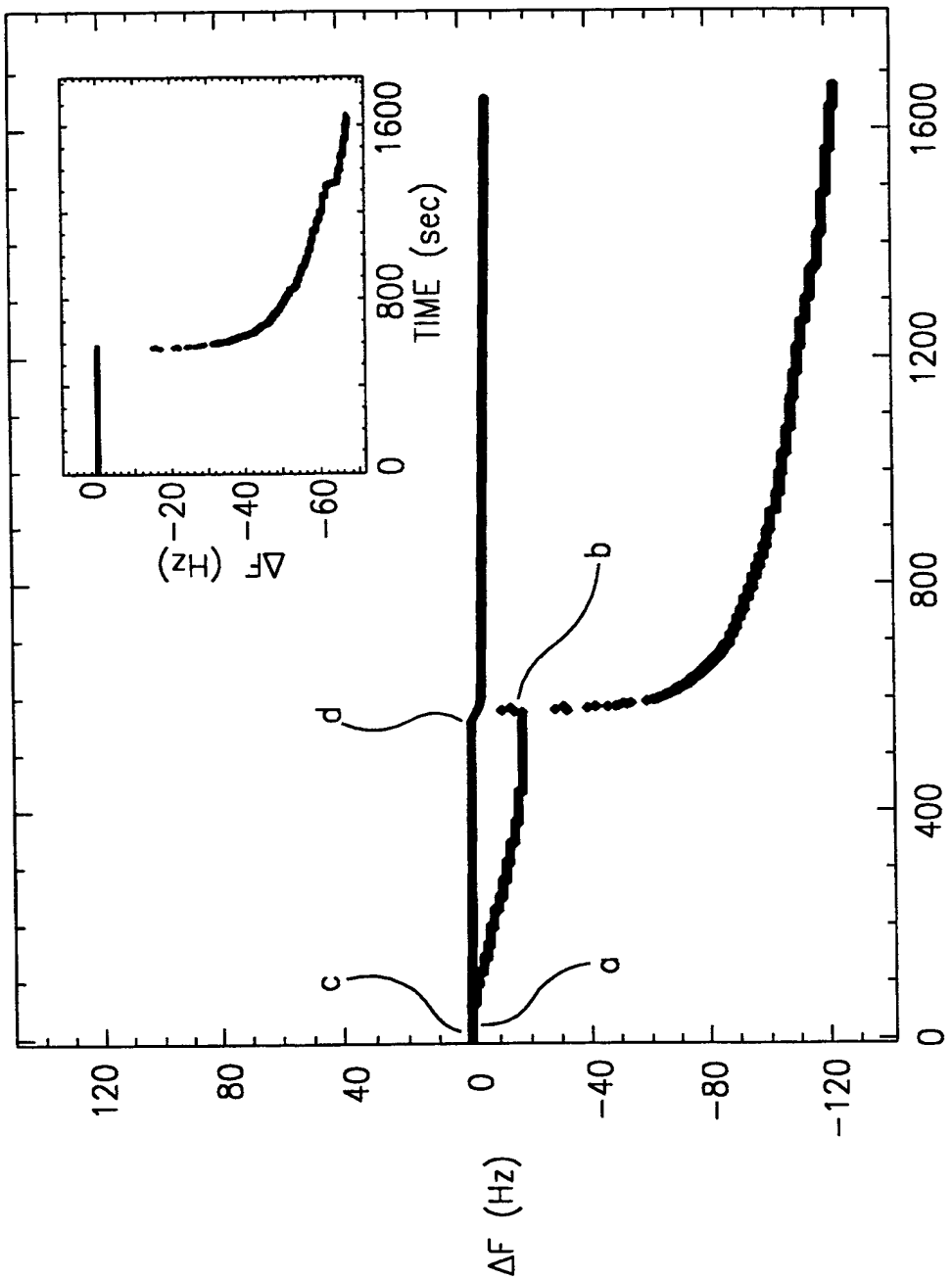


FIG.7B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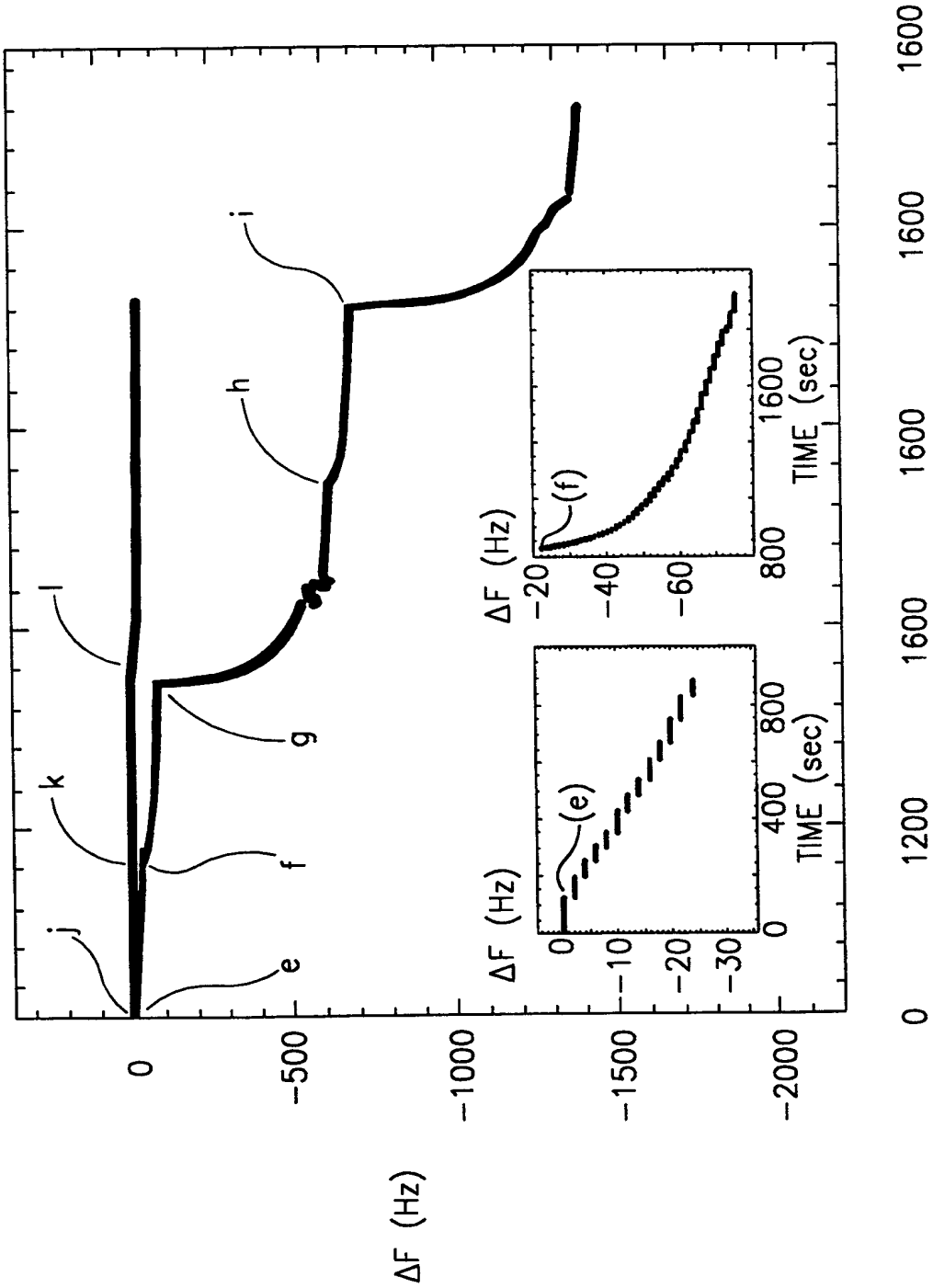
80
40
0
-40
-80
-120
0
400
800
1200
1600
TIME (sec)
a
b
c
d
ΔF (Hz)
0
-20
-40
-60
0
800
1600
TIME (sec)

FIG.8A


ΔF (Hz)
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-500
-1000
-1500
-2000
TIME (sec)
0
1200
1600
1600
1600
1600
e
f
g
h
i
j
k
l
(e)
(f)
ΔF (Hz)
TIME (sec)
0
-10
-20
-30
400
800
-20
-40
-60
800
1600

FIG. 8B

SEQUENCE LISTING

<110> YISSUM RESEARCH DEVELOPMENT COMPANY

<120> METHOD AND SYSTEM FOR DETECTING OLIGONUCLEOTIDES IN A SAMPLE

<130> 1206184 - YISSUM

<140>
<141>

<150> IL127346
<151> 1998-12-01

<150> IL 132966
<151> 1999-11-16

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<170> PatentIn Ver. 2.1

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atatgccaag cgcg 14

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 99/00649

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 695 926 A (CROS PHILIPPE ET AL) 9 December 1997 (1997-12-09) see whole doc esp. claims	1
X Y	WO 92 08808 A (SISKA DIAGNOSTICS INC) 29 May 1992 (1992-05-29) see claims and figure	1 2-7,16
Y	OKAHATA Y. ET AL.,: "Kinetic measurement of DNA hybridization on an oligonucleotide-immobilized 27-MHz quartz crystal microbalance" ANAL. CHEM., vol. 70, - 1 April 1998 (1998-04-01) pages 1288-1296, XP000891733 the whole document	2-7,16

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 April 2000	13/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	JENSEN K K ET AL: "KINETICS FOR HYBRIDIZATION OF PEPTIDE NUCLEIC ACIDS (PNA) WITH DNA AND RNA STUDIED WITH THE BIACORE TECHNIQUE" BIOCHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. EASTON, PA, vol. 36, 1 January 1997 (1997-01-01), pages 5072-5077, XP002062488 ISSN: 0006-2960	
P,X	PATOLSKY F. ET AL.,: "Enzyme-linked amplified electrochemical sensing of oligonucleotide-DNA interactions by means of the precipitation of a insoluble product and using impedance spectroscopy" LANGMUIR, vol. 15, - 29 April 1999 (1999-04-29) pages 3703-3706, XP000901187 the whole document	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 99/00649

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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